

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/70, 31/675, 31/52, A61P 31/14	A1	(11) International Publication Number: WO 00/25797 (43) International Publication Date: 11 May 2000 (11.05.00)
(21) International Application Number: PCT/US99/25673 (22) International Filing Date: 2 November 1999 (02.11.99) (30) Priority Data: 60/106,664 2 November 1998 (02.11.98) US (71) Applicant (for all designated States except US): TRIANGLE PHARMACEUTICALS, INC. [US/US]; 4 University Place, 4611 University Drive, Durham, NC 27707 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FURMAN, Phillip, A. [US/US]; 901 Bluestone Road, Durham, NC 27713 (US). PAINTER, George, R. [US/US]; 129 Red Bud Lane, Chapel Hill, NC 27514 (US). BARRY, David [US/US]; 1810 Southlakeshore Drive, Chapel Hill, NC 27515 (US). ROUSSEAU, Franck [FR/US]; 7 Sinclair Circle, Durham, NC 27705 (US). (74) Agent: KNOWLES, Sherry, M.; King & Spalding, 191 Peachtree Street, Atlanta, GA 30303-1763 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: COMBINATION THERAPY TO TREAT HEPATITIS B VIRUS (57) Abstract The present invention is directed to a method for treating hepatitis B virus infection in humans comprising administering a synergistically effective amount of agents having known anti-hepatitis B virus activity in combination or alternation. Specifically, the invention is directed to a method for treating hepatitis B virus infection comprising administering FTC in combination or alternation with penciclovir, famciclovir or Bis-POM-PMEA. Additionally, the invention is directed to a method for treating hepatitis B virus infection comprising administering L-FMAU in combination or alternation with DAPD, penciclovir or Bis-POM-PMEA. The invention is further directed to a method for treating hepatitis B virus infection comprising administering DAPD in combination or alternation with Bis-POM-PMEA.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Combination Therapy to Treat Hepatitis B Virus

This invention is in the area of methods for the treatment of hepatitis B virus (also referred to as "HBV") that includes administering to a host in need thereof, an effective
5 combination of nucleosides which have known anti-hepatitis B activity.

HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

10 Hepatitis B virus has reached epidemic levels worldwide. After a two to three month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed.

15 Patients typically recover from acute hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300
20 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is very similar to that of acquired immune deficiency syndrome (AIDS), which accounts for why
25 HBV infection is common among patients with AIDS or AIDS related complex. However, HBV is more contagious than HIV.

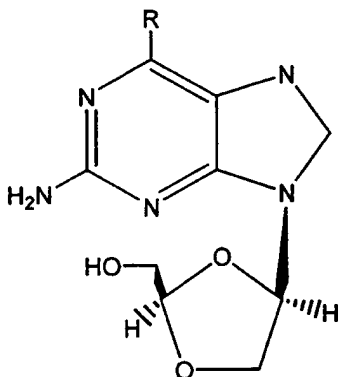
However, more recently, vaccines have also been produced through genetic engineering and are currently used widely. Unfortunately, vaccines cannot help those already infected with HBV. Daily treatments with α - interferon, a genetically engineered protein, has
30 also shown promise, but this therapy is only successful in about one third of treated patients. Further, interferon cannot be given orally.

A number of synthetic nucleosides have been identified which exhibit activity against HBV. The (-)-enantiomer of BCH-189, known as 3TC, claimed in U. S. Patent 5,539,116 to Liotta, et al., has been approved by the U. S. Food and Drug Administration for the treatment of hepatitis B. See also EPA 0 494 119 A1 filed by BioChem Pharma, Inc.

- 5 β -2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), claimed in U. S. Patent Nos. 5,814,639 and 5,914,331 to Liotta, et al., exhibits activity against HBV. See Furman, et al., "The Anti-Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (-) and (+) Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-oxathiolane-5-yl]-Cytosine" Antimicrobial Agents and Chemotherapy, December 1992, page 2686-2692; and
- 10 Cheng, et al., Journal of Biological Chemistry, Volume 267(20), 13938-13942 (1992).

U. S. Patent Nos. 5,565,438, 5,567,688 and 5,587,362 (Chu, et al.) disclose the use of 2'-fluoro-5-methyl- β -L-arabinofuranolyluridine (L-FMAU) for the treatment of hepatitis B and Epstein Barr virus.

- U. S. Patent No. 5,767,122 to Emory University and The University of Georgia
- 15 Research Foundation, Inc. discloses and claims enantiomerically pure β -D-dioxolanyl nucleosides of the formula:



- wherein R is NH₂, OH, Cl, or H. A method for treating HBV infection using a combination
- 20 of DAPD and FTC is claimed in U. S. Patent No. 5,684,010 to Raymond F. Schinazi.

Penciclovir (2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6H-purin-6-one; PCV) has established activity against hepatitis B. See U.S. Patent Nos. 5,075,445 and 5,684,153.

Adefovir (9-[2-(phosphonomethoxy)ethyl]adenine, also referred to as PMEAs or [[2-(6-amino-9H-purin-9-yl)ethoxy]methylphosphonic acid), also has established activity against hepatitis B. See for example U.S. Patent Nos. 5,641,763 and 5,142,051.

Yale University and The University of Georgia Research Foundation, Inc. disclose the use of L-FDDC (5-fluoro-3'-thia-2',3'-dideoxycytidine) for the treatment of hepatitis B virus in WO 92/18517.

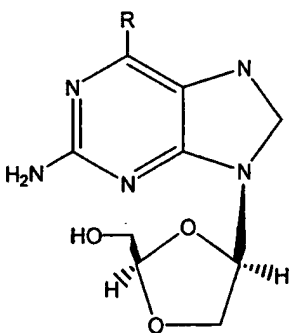
von Janta-Lipinski et al. disclose the use of the L-enantiomers of 3'-fluoro-modified β -2'-deoxyribonucleoside 5'-triphosphates for the inhibition of hepatitis B polymerases (J. Med. Chem., 1998, 41,2040-2046). Specifically, the 5'-triphosphates of 3'-deoxy-3'-fluoro- β -L-thymidine (β -L-FTTP), 2',3'-dideoxy-3'-fluoro- β -L-cytidine (β -L-FdCTP), and 2',3'-dideoxy-3'-fluoro- β -L-5-methylcytidine (β -L-FMethCTP) were disclosed as effective inhibitors of HBV DNA polymerases.

It has been recognized that drug-resistant variants of HBV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in the viral lifecycle, and most typically in the case of HBV, DNA polymerase. Recently, it has been demonstrated that the efficacy of a drug against HBV infection can be augmented by administering the compound in combination with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution, or other parameter of the drug can be altered by such combination therapy. In general, combination therapy induces multiple simultaneous stresses on the virus.

United States Patent No. 5,808,040 discloses that L-FMAU can be administered in combination with FTC, 3TC, carbovir, acyclovir, interferon, AZT, DDI (2',3'-dideoxyinosine), DDC (2',3'-dideoxycytidine), L-DDC, L-F-DDC, and D4T.

United States Patent No. 5,674,849 discloses the use of a nucleoside in combination with an oligonucleotide for the treatment of a viral disease.

United States Patent No. 5,684,010 discloses a method for the treatment of hepatitis B that includes administering in combination or alternation a compound of the formula:



wherein R is NH₂, OH, or Cl, with FTC, 3TC, carbovir, or interferon.

5 WO 98/23285 discloses a method for the treatment or prophylaxis of hepatitis B virus infections in a human or animal patient which comprises administering to the patient effective or prophylactic amounts of penciclovir (or a bioprecursor thereof such as famciclovir) and alpha-interferon.

In light of the fact that hepatitis B virus has reached epidemic levels worldwide, and
10 has severe and often tragic effects on the infected patient, there remains a strong need to provide new effective treatments for humans infected with the virus that have low toxicity to the host.

Therefore, it is an object of the present invention to provide new methods for the treatment of human patients or other hosts infected with hepatitis B virus and related
15 conditions comprising administering a synergistically effective amount of a combination of anti-HBV agents.

Summary of the Invention

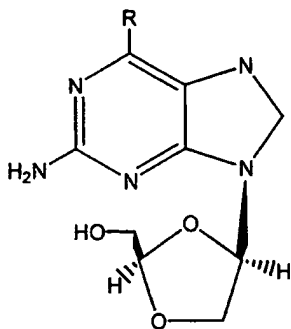
It has been discovered that certain combinations of agents with hepatitis B activity are
20 synergistic, and thus can provide enhanced benefits to the patient when administered in an effective combination or alternation dosage pattern.

In one preferred embodiment of the present invention, a method for treating HBV infection and related conditions in humans is disclosed, comprising administering a synergistically effective amount of β -2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC), preferably substantially in the form of the (-)-optical isomer, or a
25

pharmaceutically acceptable salt, ester or prodrug thereof with Penciclovir (2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6H-purin-6-one, also referred to as "PCV"). Famciclovir, or any other bioprecursor of Penciclovir, can be used in place of Penciclovir in any embodiment of this invention.

- 5 Another preferred embodiment of the present invention is a method for treating HBV infection and related conditions in humans, comprising administering in combination or alternation a synergistically effective amount of β -2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC), preferably substantially in the form of the (-)-optical isomer, or a pharmaceutically acceptable salt, ester or prodrug thereof, with 9-[2-
- 10 (phosphonomethoxy)ethyl]adenine (PMEA, also referred to below as Bis-POM-PMEA or BP-PMEA), or a pharmaceutically acceptable salt, ester or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

- In another preferred embodiment of the present invention, a method for treating HBV infection and related conditions in humans is disclosed, comprising administering in
- 15 combination or alternation a synergistically effective amount of 2'-fluoro-5-methyl- β -L-arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester or prodrug thereof, with a compound of the formula:



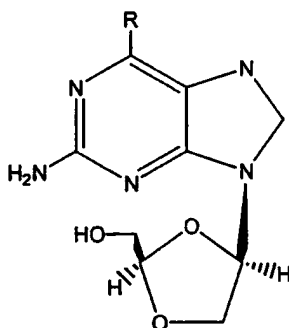
- preferably β -D-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine
- 20 (DAPD), which is preferably administered in substantially pure form, or a pharmaceutically acceptable salt, ester or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

- In yet another preferred embodiment of the present invention, a method for treating HBV infection and related conditions in humans is disclosed, comprising administering a synergistically effective combination or alternation amount of 2'-fluoro-5-methyl- β -L-
- 25 arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester or prodrug

thereof, with Penciclovir, or a pharmaceutically acceptable salt, ester or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

In still another preferred embodiment of the present invention, a method for treating HBV infection and related conditions in humans is disclosed, comprising administering a synergistically effective amount of 2'-fluoro-5-methyl- β -L-arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester or prodrug thereof, with 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), or a pharmaceutically acceptable salt, ester or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

Another preferred embodiment of the present invention comprises a method for treating HBV infection and related conditions in humans, comprising administering a synergistically effective amount of a compound of the formula:



wherein R is NH_2 , OH, H, or Cl (collectively referred to herein as the DAPD compounds), preferably, β -D-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine (DAPD), which is preferably administered in substantially pure form, or a pharmaceutically acceptable salt, ester or prodrug thereof, with PMEA, or a pharmaceutically acceptable salt, ester or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

Detailed Description of the Invention

As used herein, the term "isolated enantiomer" refers to a nucleoside composition that includes approximately 95% to 100%, or more preferably, over 97% of a single enantiomer of that nucleoside.

The terms "substantially pure form" or substantially free of its opposite enantiomer refers to a nucleoside composition of one enantiomer that includes no more than about 5% of

the other enantiomer, more preferably no more than about 2%, and most preferably less than about 1% is present.

The synergistic combination of compounds or their pharmaceutically acceptable esters or salts, are also useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These synergistic formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV antigen positive or who have been exposed to HBV.

The active compound can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agents, for example, an acid halide or anhydride. The compound or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of the compound can be converted into the parent compound, for example, by hydrolysis.

The term "synergistic combination" refers to a combination of drugs which produces a synergistic effect *in vivo*, or alternatively, *in vitro* as measured according to the methods described herein.

I. Active Compounds, and Physiological Acceptable Salts Thereof

The active compounds disclosed herein are therapeutic nucleosides or cyclic or acyclic nucleoside analogs with known activity against hepatitis B. It has been discovered that certain combinations of nucleosides provide an advantage over monotherapy, or over other combinations. Not all combinations of the known anti-HBV drugs provide a benefit; it is often the case that drugs act antagonistically.

The active compound can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5' and N⁴ cytosinyl or N⁶-adeninyl acylated (esterified) derivatives of the active compound (alternatively referred to as "physiologically active derivatives"). In one embodiment, the acyl group is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight,

branched, or cyclic alkyl or lower alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy, or is a sulfonate ester such as alkyl or aralkyl sulphonyl including methanesulfonyl, phosphate, including but not limited to
5 mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g., dimethyl-5-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optionally comprise a phenyl group.

Modifications of the active compound, and especially at the N⁴ cytosinyl or N⁶ adeninyl and 5'-O positions, can affect the bioavailability and rate of metabolism of the
10 active species, thus providing control over the delivery of the active species. Further, the modifications can affect that antiviral activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its antiviral activity according to the methods described herein, or other methods known to those skilled in the art.

15 **Prodrugs**

Any of the anti-hepatitis B agents described herein can be administered as a prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of hydroxyl-bound prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the hydroxy, mono, di or triphosphate of the
20 nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the hydroxyl or phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, **27** (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 5'-OH of the nucleoside or hydroxyl of the acyclic nucleoside analogs (such as PMEA or Penciclovir), include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin, et al.); 5,194,654 (mar. 16, 1993, Hostetler, et al.); 5,223,263 (June 29, 1993, Hostetler, et al.); 5,256,641 (Oct. 26, 1993, Yatvin, et al.);
25 5,411,947 (May 2, 1995, Hostetler, et al.); 5,463,092 (Oct. 31, 1995, Hostetler, et al.); 5,543,389 (Aug. 6, 1996, Yatvin, et al.); 5,543,390 (Aug. 6, 1996, Yatvin, et al.); 5,543,391 (Aug. 6, 1996, Yatvin, et al.); and 5,554,728 (Sep. 10, 1996, Basava, et al.).

Foreign patent applications that disclose lipophilic substituents that can be attached to the active compounds of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

5

II. Preparation of the Active Compounds

The therapeutic nucleosides used in the synergistic compositions of the present invention and processes for preparing them are known in the art.

β -2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC), and its enantiomers, can be prepared by the methods disclosed in U. S. Patent Nos. 5,204,466, 5,700,937, 5,728,575 and 5,827,727.

2'-Fluoro-5-methyl- β -L-arabinofuranolyluridine (L-FMAU) can be prepared by the methods disclosed in U. S. Patent Nos. 5,565,438, 5,567,688 and 5,587,362 to Chu, et al.

Methods for the preparation of the DAPD compounds, including (2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine (DAPD) are disclosed in U. S. Patent Nos. 5,767,122; 5,684,010; 5,444,063, and 5,179,104.

Pencyclovir can be prepared by the methods disclosed in U.S. Patent Nos. 5,075,445 and 5,684,153.

PMEA can be prepared by the methods disclosed in U.S. Patent Nos. 5,641,763 and 5,142,051.

Mono, di, and triphosphate derivatives of the active nucleosides can be prepared as described according to published methods. The monophosphate can be prepared according to the procedure of Imai, et al., J. Org. Chem., 34(6), 1547-1550 (June 1969). The diphosphate can be prepared according to the procedure of Davisson, et al., J. Org. Chem., 52(9), 1794-1801 (1987). The triphosphate can be prepared according to the procedure of Hoard, et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965).

III. Combination Therapy

It has been recognized that drug-resistant variants of HBV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in the viral lifecycle, and most typically in the case of HBV, DNA polymerase. Recently, it has been demonstrated that the efficacy of a drug

against HBV infection can be prolonged, augmented, or restored by administering the compound in combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution, or other parameter of the drug can be altered by such combination therapy. In general, combination therapy induces multiple simultaneous stresses on the virus.

Example 1

Test compounds: DAPD, DXG, (-)- β -FTC, L-FMAU
10 DMVI assay controls: Untreated cells, 3TC (lamivudine), penciclovir (PCV)

Details of the assay methodology can be found in: Korba and Gerin, Antiviral Res. 19: 55-70 (1992) and Korba, Antiviral Res. 29: 49-52 (1996). The antiviral evaluations were performed on six separate cultures per each of four test concentrations. All wells, in all plates, were seeded at the same density and at the same time.

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.0-fold for HBV virion DNA from the average levels for these HBV DNA forms in untreated cells are generally considered to be statistically significant [$P < 0.05$] (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). Typical values for extracellular HBV virion DNA in untreated cells range from 80 to 150 pg/ml culture medium (average of approximately 92 pg/ml).

For reference, the manner in which the hybridization analyses were performed for these experiments results in an equivalence of approximately 1.0 pg of extracellular HBV DNA/ml culture medium to 3×10^5 viral particles/ml.

Toxicity analyses were performed in order to access whether any observed antiviral effects are due to a general effect on cell viability. The method used was uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV and HIV. Details of the procedure are provided in the toxicity table legends.

30 EXPERIMENTAL PARAMETERS

Test compounds were received as solid material at room temperature in good package condition. Test compounds were solubilized in 100% tissue culture grade DMSO (Sigma,

Corp.) at 100mM (DAPD, FTC, L-FMAU) or 50mM (DXG). Daily aliquots of test compounds were made in individual tubes and stored at -20°C. On each day of treatment, daily aliquots of the test compounds were suspended into culture medium at room temperature, and immediately added to the cell cultures.

- 5 For the antiviral test analyses, confluent cultures were maintained on 96-well flat bottomed tissue culture plates. Two separate (replicate) plates were used for each drug treatment. A total of 3 cultures on each plate were treated with each of the dilutions of antiviral agents (6 cultures per dilution). Cultures were treated with 9 consecutive daily doses of the test compounds. Medium was changed daily with fresh test compounds. Only
10 extracellular (virion) HBV DNA levels were followed.

- Toxicity analysis were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule and under identical culture conditions as used for the antiviral evaluations. Each compound was tested at 4 concentrations, each in triplicate cultures. Uptake of neutral red
15 dye was used to determine the relative level of toxicity 24 hours following the last treatment. The absorbance of internalized dye at 510 nM (A_{510}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{510} values (\pm standard deviations) in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds.

- 20 Combination treatments were conducted using the primary analysis format except that 6 serial 3-fold dilutions were used for each drug combination and a total of 8 separate cultures were used for each dilution of the combinations. Compounds were mixed at molar ratios designed to give approximately equipotent antiviral effects based on the EC_{90} values. Three different molar ratios were used for each combination to allow for variability in the
25 estimates of relative potency. These molar ratios were maintained throughout the dilution series. The corresponding monotherapies were conducted in parallel to the combination treatments using the standard primary assay format.

- For reporting purposes, the SI, EC_{50} , EC_{90} , and CC_{50} values reported for the combination treatments are those of the first compound listed for the combination mixture.
30 The concentrations and SI, EC_{50} , EC_{90} , and CC_{50} values of the second compound in the mixture can be calculated using the molar ratio designated for that particular mixture.

Further details on the design of combination analyses as conducted for this report can be found in BE Korba (1996) *Antiviral Res.* 29:49.

Analysis of synergism, additivity, or antagonism were determined by analysis of the data using the CalcuSynTM program (Biosoft, Inc.). This program evaluates drug interactions by use of the widely accepted method of Chou and Talalay combined with a statistically evaluation using the Monte Carlo statistical package. The data are displayed in several different formats including median-effect and dose-effects plots, isobolograms, and combination index [CI] plots with standard deviations. For the latter analysis, a CI greater than 1.0 indicates antagonism and a CI less than 1.0 indicates synergism.

For the toxicity analyses associated with the combination treatments, the experimental design was limited by either/or the toxicity of the more toxic compound in the mixture or the stock concentrations (e.g. related to the total volume of DMSO that could be added to the cultures without inducing toxicity due to DMSO and not the test compounds).

Antiviral Evaluations

ASSAY CONTROLS: Within normal variations, levels of extracellular HBV (virion) DNA remained constant in the untreated cells over the challenge period. The positive treatment controls, 3TC (lamivudine) [((-)- β ,L,2',3'-dideoxy-3' thiacytidine] and penciclovir [PCV] (both purchased from Moraveck Biochemicals, La Brea, CA), induced significant depressions of HBV DNA replication at the concentrations used. The activities observed for 3TC in these analyses were consistent with previous experiments where approximately 0.15 to 0.2 μ M 3TC induced a 90% depression of HBV virion DNA relative to average levels in untreated cells after 9 days of continuous treatment of 2.2.15 cells [EC₉₀] (for example, see Korba and Boyd, *Antimicrob. Agents Chemother.* (1996) 40:1282-1284). The activities observed for PCV in these analyses were higher than previously reported (EC₉₀ of approximately 0.7 to 0.9 μ M, Korba and Boyd, *Antimicrob. Agents Chemother.* (1996) 40:1282-1284). However, the preparation of PCV used for these experiments has consistently produced anti-HBV activities in the range reported here in several other independent experiments.

TEST COMPOUNDS: Test compound DAPD, FTC, DXG, and L-FMAU induced significant and selective depressions in extracellular (virion) HBV DNA levels produced by 2.2.15 cells.

The antiviral activity of DAPD was enhanced by co-treatment with FTC. The antiviral activity of DAPD was synergistic at a 3:1 or a 1:1 molar ratio at all but the highest concentrations tested. As the relative concentration of FTC increased, the co-operative effects of the two agents decreased. At the 1:3 molar ratio, the two agents appeared to be
5 antagonistic.

DAPD and PCV appeared to be antagonistic at all three molar ratios and at all concentrations.

At the 1:10 and 1:1 molar ratios, DAPD and L-FMAU appeared to be antagonistic. At the 1:3 molar ratio (approximately equipotent potencies based on the EC₉₀'s) the
10 interactions of the two agents were more complex. DAPD and L-FMAU exhibited moderately synergistic to additive interactions at lower concentrations which progressed to increasingly more antagonistic interactions at higher concentrations. Subsequent testing, however, indicated that DAPD is synergistic with L-FMAU.

The antiviral activity of L-FMAU was enhanced by co-treatment with FTC. The
15 antiviral activity of DAPD and FTC was moderately synergistic at a 3:1 or a 10:1 molar ratio at all but the highest concentrations tested. As the relative concentration of FTC increased, the cooperative effects of the two agents decreased. At the 1:1 molar ratio, the two agents appeared to be antagonistic.

The antiviral activity of L-FMAU was also enhanced by co-treatment with PCV. The
20 antiviral activity of DAPD and PCV was weakly synergistic at a 1:1 or a 1:3 molar ratio at all concentrations tested. As the relative concentration of PCV increased, the co-operative effects of the two agents decreased. At the 1:10 molar ratio, the two agents appeared to be antagonistic.

25 **Toxicity Evaluations**

No significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for 3TC, PCV, or any of the test compounds at the concentrations used for the antiviral evaluations.

None of the combination treatments appeared to enhance the toxicity profiles of either
30 agent in the different mixtures. The toxicity profiles of some of the combination mixtures was apparently higher than the corresponding monotherapies since the values are reported as a factor of the concentration of the first compound listed for each mixture. This is especially

notable for the mixtures containing PCV. However, recalculation of the toxicity profiles on the basis of the second compound (e.g. PCV) in the mixtures revealed that all of the apparent toxicities were due to the more toxic compound and that no enhanced toxicity was present in these combinations.

5

Example 2

Test compounds provided: (-)- β -FTC

DMVI assay controls: untreated cells, 3TC (lamivudine), penciclovir (PCV)

10 Details of the assay methodology were as given above. Test compounds were received as solid material at room temperature in good package condition. Test compounds were solubilized in 100% tissue culture grade DMSO (Sigma, Corp.) at 100mM. Daily aliquots of test compounds were made in individual tubes and stored at -20°C

TEST COMPOUNDS: Test compound FTC induced significant and selective
15 depressions in extracellular (virion) HBV DNA levels produced by 2.2.15 cells.

The antiviral activity of FTC was enhanced by co-treatment with PCV. The antiviral activity of the combination therapy was synergistic at all molar ratios tested. As the relative concentration of PCV increased, the cooperative effects of the two agents decreased.

20 Toxicity Evaluations

No significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for 3TC, PCV, FTC, or any of the combination treatments at the concentrations used for the antiviral evaluations (Tables S1, T1).

None of the combination treatments appeared to enhance the toxicity profiles of either
25 agent in the different mixtures. The toxicity profiles of some of the combination mixtures was apparently higher than the corresponding monotherapies since the values are reported as a factor of the concentration of the first compound listed for each mixture.

Example 3 Combination Therapy with PME A

Test compounds provided: PME A, (-)- β -FTC, DAPD, L-FMAU

DMVI assay controls: Untreated cells, 3TC (lamivudine)

5

Details of the assay methodology were as given above. Test compounds (except for bis-POM-PME A) were received as powdered material on dry ice in good package condition and stored at -20°C. Test compound bis-POM-PME A was received as a 100mM solution in DMSO. Daily aliquots of test compounds were made in individual tubes and stored at -20°C.

- 10 On each day of treatment, daily aliquots of the test compounds were suspended into culture medium at room temperature, and immediately added to the cell cultures.

TEST COMPOUNDS (PRIMARY ANALYSES): All of the test compounds induced significant and selective depressions in extracellular (virion) HBV DNA levels produced by 2.215 cells. However, the potencies of test compounds (-)- β -FTC, DAPD and L-FMAU were
15 lower than that observed in earlier analyses. This was most apparent for DAPD and L-FMAU.

Bis-POM-PME A (BP-PME A) + FTC. The mixture of BP-PME A and FTC produced an anti-HBV activity that was moderately synergistic overall. The potency of the mixtures increased as the relative proportion of FTC increased. However, the most favorable
20 overall interactions occurred where the concentration of FTC was proportionately lower. The same relative degree of synergism was generally observed at all concentrations of the 30:1 mixture. Relatively more synergistic interactions were observed at the lower concentrations of the 10:1 and 3:1 mixture and moderate to strong antagonism was observed at the highest concentrations of the 3:1 mixture.

25 **BP-PME A + DAPD.** The mixture of BP-PME A and DAPD produced an anti-HBV activity that was moderately to weakly synergistic at lower relative concentrations of DAPD and moderately to strongly antagonistic at higher relative concentrations of DAPD. The potency of the mixtures also decreased as the relative proportion of DAPD increased. Relatively more synergistic interactions were observe at the lower concentrations of the
30 different mixtures.

BP-PME A + L-FMAU. The mixture of BP-PME A and L-FMAU produced an anti-HBV activity that was moderately synergistic at lower relative concentrations of L-FMAU

and additive to weakly antagonistic at higher relative concentrations of L-FMAU. The potency of the mixtures was lowest at the highest relative concentration L-FMAU (1:1 molar ratio). The most favorable overall interactions were observed at the 3:1 molar ratio of the two compounds. Relatively more synergistic interactions were observed at the lower concentrations of different mixtures.

Toxicity Evaluations

No significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for 3TC, any of the test compounds, or any of the compound mixtures at the concentrations used for the antiviral evaluations. None of the compound mixtures appeared to significantly enhance toxicity. The patterns of toxicity observed for the compound mixtures was similar to, and consistent with, that observed for the monotherapies.

IV. Preparation of Pharmaceutical Compositions

Humans suffering from any of the diseases described herein arising out of HBV infection, can be treated by administering to the patient an effective amount of identified synergistic anti-HBV agents in a combination or independent dosage form for combination or alternation therapy, optionally in a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compounds are included in the pharmaceutically acceptable carriers or diluents in amounts sufficient to deliver to a patient a therapeutically effective amount of compound to inhibit viral replication *in vivo*, especially HBV replication, without causing serious toxic effects in the patient treated. By "inhibitory amount" is meant an amount of active ingredient sufficient to exert an inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of the compound for all the above-mentioned conditions will be in the range from about 1 to 50 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in

itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The compound is conveniently administered in unit or any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. An oral dosage of 50-1000 mg is usually convenient, more typically 50-300 mg.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μ M, preferably about 1.0 to 10 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a

flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The compound can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The compound or a pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, protease inhibitors, or other nucleoside or nonnucleoside antiviral agents, as discussed in more detail above. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation.

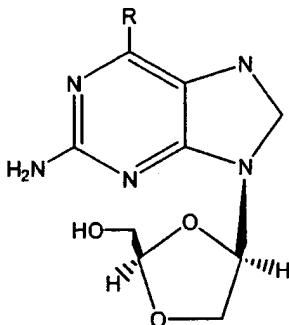
Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable

carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadoyl phosphatidyl choline, and
5 cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

10 This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of this invention.

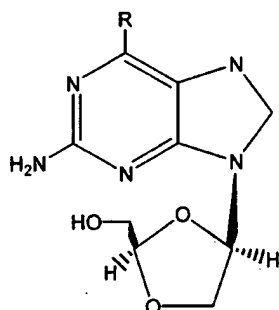
We Claim:

1. A method for the treatment of hepatitis B virus in a human comprising administering in combination or alternation a synergistically effective amount of β -2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (β -FTC) or a pharmaceutically acceptable salt, ester, or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the group consisting of penciclovir, famciclovir, and Bis-POM-PMEA.
2. The method of claim 1, wherein the β -FTC is in the form of the (-)-optical isomer.
3. The method of claim 2, wherein the second anti-hepatitis B agent is penciclovir.
4. The method of claim 2, wherein the second anti-hepatitis B agent is famciclovir.
5. The method of claim 2, wherein the second anti-hepatitis B agent is Bis-POM-PMEA.
6. A method for the treatment of hepatitis B virus in a human comprising administering in combination or alternation a synergistically effective amount of 2'-fluoro-5-methyl- β -L-arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester, or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the group consisting of a compound of the formula



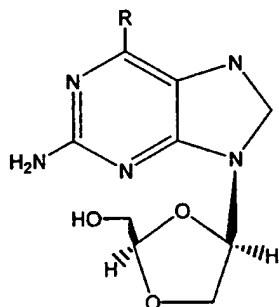
wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof, penciclovir, and 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), or a pharmaceutically acceptable salt, ester or prodrug thereof.

7. The method of claim 6, wherein the second anti-hepatitis B agent is a compound of the formula



wherein R is OH, or a pharmaceutically acceptable salt, ester or prodrug thereof.

5 8. The method of claim 6, wherein the second anti-hepatitis B agent is a compound of the formula

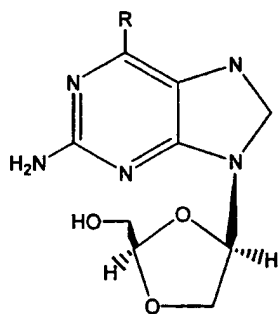


wherein R is NH₂, or a pharmaceutically acceptable salt, ester or prodrug thereof.

9. The method of claim 6, wherein the second anti-hepatitis B agent is
10 penciclovir.

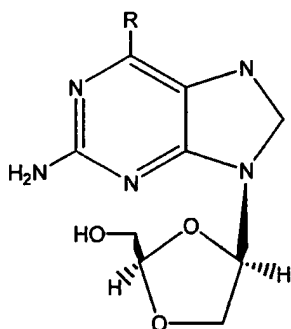
10. The method of claim 6, wherein the second anti-hepatitis B agent is PME₃, or a pharmaceutically acceptable salt, ester or prodrug thereof.

11. A method for the treatment of hepatitis B virus in a human comprising administering in combination or alternation a synergistically effective amount of a compound
15 of the formula



wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the group consisting of PMEAs, or a pharmaceutically acceptable salt, ester or prodrug thereof.

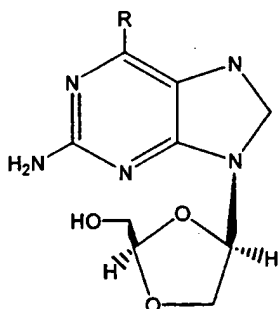
- 5 12. The method of claim 11 wherein R is OH.
13. The method of claim 11 wherein R is NH₂.
14. Use of a synergistically effective amount of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (β-FTC) or a pharmaceutically acceptable salt, ester, or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the
- 10 group consisting of penciclovir, famciclovir, and Bis-POM-PMEA for the treatment of hepatitis B virus.
15. Use of a synergistically effective amount of 2'-fluoro-5-methyl-β-L-arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester, or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the group
- 15 consisting of a compound of the formula



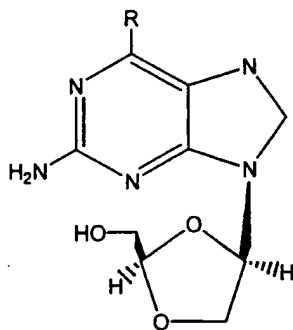
wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof, penciclovir, and 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), or a

pharmaceutically acceptable salt, ester or prodrug thereof for the treatment of hepatitis B virus.

16. Use of a synergistically effective amount of a compound of the formula

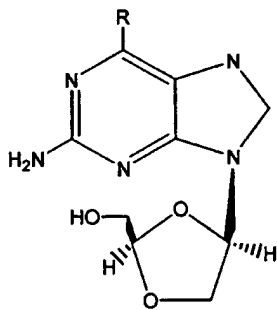


- 5 wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof with an effective amount of a second anti-hepatitis B agent selected from the group consisting of PME_A, or a pharmaceutically acceptable salt, ester or prodrug thereof for the treatment of hepatitis B virus.
- 10 17. A pharmaceutical composition comprising an effective amount of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (β-FTC) or a pharmaceutically acceptable salt, ester, or prodrug thereof in a synergistic combination with an effective amount of a second anti-hepatitis B agent selected from the group consisting of penciclovir, famciclovir, and Bis-POM-PME_A.
- 15 18. The composition of claim 17, wherein the second anti-hepatitis B agent is penciclovir.
19. The composition of claim 17, wherein the second anti-hepatitis B agent is famciclovir.
20. The composition of claim 17, wherein the second anti-hepatitis agent is Bis-POM-PME_A.
- 20 21. A pharmaceutical composition comprising an effective amount of 2'-fluoro-5-methyl-β-L-arabinofuranolyluridine (L-FMAU), or a pharmaceutically acceptable salt, ester, or prodrug thereof in a synergistic combination with an effective amount of a second anti-hepatitis B agent selected from the group consisting of a compound of the formula



wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof, penciclovir, and 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), or a pharmaceutically acceptable salt, ester or prodrug thereof.

- 5 22. The composition of claim 21, wherein R is NH₂.
23. The composition of claim 21, wherein R is OH.
24. A pharmaceutical composition comprising an effective amount of a compound of the formula



- 10 wherein R is NH₂, OH, Cl, or H, or a pharmaceutically acceptable salt, ester or prodrug thereof in a synergistic combination with an effective amount of a second anti-hepatitis B agent selected from the group consisting of PMEA, or a pharmaceutically acceptable salt, ester or prodrug thereof.

25. The composition of claim 24, wherein R is NH₂.
- 15 26. The composition of claim 24, wherein R is OH.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/25673

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/70 A61K31/675 A61K31/52 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 23285 A (SMITHKLINE BEECHAM PLC) 4 June 1998 (1998-06-04) cited in the application claims 1-14	1-24
A	WO 94 09793 A (EMORY UNIVERSITY) 11 May 1994 (1994-05-11) claims 13-24	1-24
A	MIGYOUNG L ET AL: "Dioxolane cytosine nucleosides as anti-hepatitis B agents" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, GB, OXFORD, vol. 5, no. 17, 7 September 1995 (1995-09-07), pages 2011-2014, XP004135355 ISSN: 0960-894X the whole document	1-24

-/-

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

5 April 2000

Date of mailing of the international search report

12/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Siatou, E

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/25673

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 494 119 A (IAF BIOCHEM INT) 8 July 1992 (1992-07-08) cited in the application	1-24
A	WO 95 20595 A (UNIV GEORGIA ;UNIV YALE (US); CHU CHUNG K (US); CHENG YUNG CHI (US) 3 August 1995 (1995-08-03) & US 5 565 438 A cited in the application	1-24
A	EP 0 141 927 A (BEECHAM GROUP PLC) 22 May 1985 (1985-05-22) & US 5 075 445 A cited in the application	1-24
A	EP 0 253 412 A (REGA FOUNDATION ;CESKOSLOVENSKA AKADEMIE VED (CS)) 20 January 1988 (1988-01-20) & US 5 641 763 A cited in the application	1-24

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/25673

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-13
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 14, 17-20

Treatment of Hepatitis B by a synergistic combination of beta-FTC and a second anti-HVB agent selected from penciclovir, famciclovir, bis-POM-PMEA

2. Claims: 6-10, 15, 21-23

Treatment of hepatitis B by administering a combination of L-FMAU and a second HVB agent selected from penciclovir, PMEA, dioxolanyl nucleoside derivatives

3. Claims: 11-13, 16, 24-26

Treatment of hepatitis B by administration of a dioxolanyl nucleoside derivative and a second anti-HVB agent selected from PMEA, salts or prodrugs thereof

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/25673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9823285 A	04-06-1998	AU 5127098 A	22-06-1998
WO 9409793 A	11-05-1994	US 5444063 A	22-08-1995
		AT 165004 T	15-05-1998
		AU 693079 B	25-06-1998
		AU 5541994 A	24-05-1994
		CA 2147893 A	11-05-1994
		CN 1092427 A, B	21-09-1994
		CN 1152435 A	25-06-1997
		DE 69318031 D	20-05-1998
		DE 69318031 T	03-09-1998
		EP 0666749 A	16-08-1995
		ES 2115918 T	01-07-1998
		JP 8507286 T	06-08-1996
		US 5684010 A	04-11-1997
		US 5830898 A	03-11-1998
		US 5834474 A	10-11-1998
EP 0494119 A	08-07-1992	AT 120644 T	15-04-1995
		AU 660650 B	06-07-1995
		AU 1153492 A	17-08-1992
		CA 2100269 A, C	04-07-1992
		CA 2254613 A	04-07-1992
		WO 9211852 A	23-07-1992
		CY 2047 A	30-04-1998
		DE 69201948 D	11-05-1995
		DE 69201948 T	03-08-1995
		DK 565549 T	03-07-1995
		EP 0565549 A	20-10-1993
		ES 2070628 T	01-06-1995
		GR 3015694 T	31-07-1995
		HK 159395 A	20-10-1995
		IE 73642 B	18-06-1997
		IL 100502 A	08-12-1995
		JP 2659863 B	30-09-1997
		JP 6507150 T	11-08-1994
		KR 156747 B	16-11-1998
		MD 950086 A	31-01-1997
		NO 180407 B	06-01-1997
		US 5532246 A	02-07-1996
WO 9520595 A	03-08-1995	US 5587362 A	24-12-1996
		AU 710262 B	16-09-1999
		AU 1737695 A	15-08-1995
		BG 100792 A	31-03-1997
		BR 9506596 A	09-09-1997
		CA 2182273 A	03-08-1995
		CN 1143966 A, B	26-02-1997
		CZ 9602114 A	16-09-1998
		EP 0748330 A	18-12-1996
		FI 962986 A	26-07-1996
		HU 75514 A	28-05-1997
		JP 9508394 T	26-08-1997
		NO 963138 A	26-09-1996
		NZ 281058 A	26-06-1998
		SK 92696 A	06-08-1997
		US 5565438 A	15-10-1996
		US 5567688 A	22-10-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/25673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0141927 A	22-05-1985	AU 577303 B	22-09-1988
		AU 3197384 A	21-02-1985
		BG 61325 B	30-05-1997
		CA 1339818 A	14-04-1998
		CZ 283182 B	14-01-1998
		CY 1746 A	03-06-1994
		DE 3485225 A	05-12-1991
		ES 535283 D	01-12-1985
		ES 8602791 A	16-03-1986
		ES 543213 D	01-01-1986
		ES 8603887 A	16-05-1986
		ES 543214 D	01-01-1986
		ES 8603888 A	16-05-1986
		GR 80121 A	17-12-1984
		HK 126093 A	19-11-1993
		IE 58334 B	08-09-1993
		JP 2519882 B	31-07-1996
		JP 60058982 A	05-04-1985
		JP 2543822 B	16-10-1996
		JP 6293764 A	21-10-1994
		MX 160431 A	22-02-1990
		NZ 209248 A	08-01-1988
		SG 114493 G	21-01-1994
		US 5075445 A	24-12-1991
		US 5886215 A	23-03-1999
		ZA 8406374 A	28-08-1985
EP 0253412 A	20-01-1988	CS 8605469 A	16-08-1988
		AT 57932 T	15-11-1990
		AU 600002 B	02-08-1990
		AU 7575987 A	21-01-1988
		DK 373487 A	19-01-1988
		EG 18273 A	30-12-1992
		FI 873165 A, B,	19-01-1988
		GR 3002534 T	25-01-1993
		IE 60117 B	01-06-1994
		IL 83235 A	15-11-1992
		JP 2114114 C	06-12-1996
		JP 8022866 B	06-03-1996
		JP 63045289 A	26-02-1988
		KR 9504179 B	27-04-1995
		LU 90155 A	22-12-1997
		NZ 221100 A	25-09-1991
		PT 85354 A, B	01-08-1987
		US 5142051 A	25-08-1992
		US 5641763 A	24-06-1997
		US 5869467 A	09-02-1999
		ZA 8705283 A	30-03-1988